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QIAseq™ CleanStart PCR Kit Handbook

For specialized PCR amplification of Illumina®-compatible NGS libraries for decreased contaminations

QIAGEN

Contents

Kit Contents

QIAseq CleanStart PCR Kit		**(100)**
Catalog no.		**180795**
Number of reactions		**100**
CleanStart PCR Mix, 2x	Red cap	2 x 1.4 ml
CleanStart PCR Primer Mix	Clear cap	1 x 150 µl
RNase-Free Water	Clear cap	3 x 1.9 ml
Quick-Start Protocol		1

Storage

The QIAseq CleanStart PCR Kit (cat. no. 180795) should be stored immediately upon receipt at –15°C to –30°C. If stored under these conditions, the kit contents are stable until the date indicated on the box label.

Intended Use

The QIAseq CleanStart PCR Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAseq CleanStart PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction and Principle

The CleanStart PCR Mix, included in the QIAseq CleanStart PCR Kit, efficiently amplifies the generated RNA-seq library, irrespective of high GC or AT content and also degenerates contaminating material, such as previously generated NGS libraries. The CleanStart PCR Mix accomplishes this by modifying the ends of generated NGS libraries during amplification. This does not affect the NGS sequencing run, but allows the specific removal of these libraries in subsequent CleanStart amplifications during the decontamination step. The CleanStart PCR Mix amplifies any Illumina-compatible QIAseq Stranded RNA library to decrease contaminations.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

- Nuclease-free pipet tips and tubes
- Nuclease-free microcentrifuge tubes
- Nuclease-free 0.2 ml PCR tubes, 96-well PCR plates or PCR strips and caps
- 1.5 ml LoBind® tubes (Eppendorf, cat. no. 022431021)
- Ice
- Microcentrifuge
- Thermal cycler
- Multichannel pipets
- Single-channel pipets
- Vortexer
- Magnetic separation stand for 1.5 ml or 2 ml tubes, e.g., DynaMag™-2 (ThermoFisher Scientific, cat. no. 12321D) or equivalent
- For optional SPRI bead purification procedure on page 9: Agencourt® AMPure® XP Beads (Beckman Coulter, cat. no. A63880)

Protocol: CleanStart PCR Amplification

This protocol describes the CleanStart PCR amplification of any Illumina-compatible QIAseq Stranded RNA NGS library to decrease contamination. A universal primer pair is used for amplification.

Important points before starting

- QIAseq CleanStart PCR reagents use a proprietary amplification reaction, in conjunction with modification enzymes, to ensure that previously constructed NGS libraries are removed. If a previously amplified CleanStart library needs to be re-amplified, for instance, when an additional library is needed to replace a failed NGS run, omit the decontamination step of the PCR protocol to disable selective degradation.
- Ensure that the reactions are thoroughly mixed by pipetting up and down, and that they are set up and incubated at the recommended temperatures.
- CleanStart PCR Mix, 2x should be thawed on ice. All other components can be thawed at room temperature (15–25°C) and stored on ice during protocol preparations. Ligation Initiator should be thawed and kept at room temperature until protocol preparations are finished. Then it should be stored at –15 to –30°C.
- Use a thermal cycler with a heated lid to incubate the reactions.

CleanStart PCR amplification

1. Add 1.5 µl CleanStart PCR Primer Mix and 25 µl CleanStart PCR Mix, 2x to each 23.5 µl sample. Briefly centrifuge, mix by pipetting up and down 10 times and centrifuge briefly again.

   Note: The 23.5 µl sample can be any Illumina-compatible QIAseq Stranded RNA NGS library.

2. Perform the amplification as described in Table 1. Select the number of PCR cycles according to your sample type and amount from Table 2.

Table 1. CleanStart library amplification cycling conditions.

Step	Time	Temperature	Number of cycles
CleanStart decontamination*	15 min	37°C	1
Initial denaturation	2 min	98°C	1
PCR	20 s 30 s 30 s	98°C 60°C 72°C	Variable†
Final extension	1 min	72°C	1
Hold	∞	4°C	Hold

* For the re-amplification of libraries, omit the CleanStart decontamination step and start with incubation at 98°C for 2 min.

† Low-quality RNA (e.g., FFPE RNA) could require additional PCR cycles. See Table 2 for details.

Table 2. Recommended number of PCR cycles for various sample inputs and amounts.

Total RNA		RNA from mRNA enrichment protocol	
Input*	Number of cycles	Input†	Number of cycles
1 ng	14–16	100 ng	14–16
10 ng	11–13	500 ng	11–13
50 ng	9–11	1 µg	9–11
100 ng	7–9	5 µg	7–9

* Indicates the amount of RNA previously enriched for mRNA or depleted for rRNA. These cycle recommendations have been determined using the QIAseq Stranded Total RNA Kit and might require optimization for other stranded library kits.

† Indicates the amount of total RNA prior to mRNA enrichment.

Note: Amplified libraries can be stored –15 to –30°C in a constant-temperature freezer for prolonged periods. Please note that after PCR, these libraries will still contain excess primers, which will interfere with sequencing. We recommend purifying libraries according to the NGS kit manufacturer's specifications.

SPRI bead purification (optional)

1. After amplification, add 50 µl Agencourt AMPure XP Beads (Beckman Coulter, cat. no. A63880) to each reaction. Mix well by pipetting up and down 10 times.
2. Incubate for 5 min at room temperature (15–25°C).
3. Place the tubes onto a magnetic rack. After the solution has cleared (~10 min or longer), carefully remove and discard the supernatant.

   Important: Do not discard the beads, as they contain the DNA of interest.
4. With the tubes still on the magnetic stand, add 200 µl of 80% ethanol. Rotate the tubes 2–3 times to wash the beads. Carefully remove and discard the wash.
5. Repeat the ethanol wash.

   Important: Completely remove all traces of the ethanol wash after this second wash. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol.
6. With the tubes still on the magnetic stand and the caps open, air dry at room temperature (15–25°C) for 5–10 min.

   Note: Visually inspect that the pellet is completely dry and that all residual ethanol is evaporated, but be careful not to over dry the beads, as this will significantly decrease elution efficiency.
7. Remove the tubes from the magnetic stand and elute the DNA from the beads by adding 22 µl nuclease-free water. Mix well by pipetting.
8. Return the tubes to the magnetic rack until the solution has cleared.
9. Transfer 20 µl of cleared sample to clean tubes/plate. Alternatively, store the samples at –15 to –30°C in a constant-temperature freezer.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit **www.qiagen.com**).

	Comments and suggestions
Low library yields	
Sub-optimal PCR cycle number	An increased number of PCR cycles in the CleanStart PCR enrichment step can increase library yields.
Bioanalyzer peaks at higher molecular weight (>1000 bp); PCR overamplification	
Reduce PCR cycle number in the CleanStart PCR enrichment step	Single-stranded library products can self-anneal after too many PCR cycles when free PCR primers are no longer available. Reduced PCR cycle numbers are only necessary when the molarity of the high molecular peak is significantly elevated (>50 % compared to library yields <700 bp).

Ordering Information

Product	Contents	Cat. no.
QIAseq CleanStart PCR Kit (100)	For PCR amplification of Illumina-compatible QIAseq Stranded NGS libraries; includes specialized CleanStart formulation to significantly decrease NGS library contamination from previous experiments	180795
Related products		
QIAseq Library Quant Array Kit	qPCR arrays for quantifying Illumina libraries	333304
QIAseq Library Quant Assay Kit	qPCR assays for quantifying Illumina libraries	333314
QIAseq Stranded Total RNA Lib Kit (24)	For 24 Stranded RNA-seq sequencing library prep reactions: fragmentation, reverse transcription, second strand synthesis + end-repair + A-addition, adapter ligation, CleanStart PCR enrichment and QIAseq Beads for library cleanups	180743
QIAseq Stranded Total RNA Lib Kit (96)	For 96 Stranded RNA-seq sequencing library prep reactions: fragmentation, reverse transcription, second strand synthesis + end-repair + A-addition, adapter ligation, CleanStart PCR enrichment and QIAseq Beads for library cleanups	180745

Product	Contents	Cat. no.
QIAseq Stranded mRNA Select Kit (24)	For 24 Stranded RNA-seq sequencing library prep reactions: mRNA enrichment of total RNA, fragmentation, reverse transcription, second strand synthesis + end-repair + A-addition, adapter ligation, CleanStart PCR enrichment and QIAseq Beads for library cleanups	180773
QIAseq Stranded mRNA Select Kit (96)	For 96 Stranded RNA-seq sequencing library prep reactions: mRNA enrichment of total RNA, fragmentation, reverse transcription, second strand synthesis + end-repair + A-addition, adapter ligation, CleanStart PCR enrichment and QIAseq Beads for library cleanups	180775

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Notes

Limited License Agreement for the QIAseq CleanStart PCR Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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